

## Biology and Clinical Uses of Erythropoietin in Infants and Children

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Erythropoietin (Epo) is a true hematopoietic hormone produced in response to lowered oxygen tension. The liver is the main source of Epo during most of fetal life, but the liver to kidney switch occurs around birth so that 90% of Epo synthesis takes place in the kidney very soon in life. Elevated serum Epo levels are observed at birth in the case of maternal hypertension, alcoholism, or diabetes mellitus, in the presence of Rh immunization or severe growth retardation, or after  $\beta$ -sympathomimetic tocolysis, all situations associated with fetal hypoxia. On the other hand, Epo production may be found to be inadequate for the degree of anemia in a variety of conditions, including renal failure, juvenile rheumatoid arthritis, acquired immune deficiency syndrome, surgery, cancer, chemotherapy, marrow transplantation, and prematurity. The pathophysiology of the anemia of prematurity is complex, but the most prominent feature is defective Epo synthesis in response to the anemia. In view of the risks associated with blood transfusions, recombinant human erythropoietin (rHuEpo) has been proposed to treat the anemia of prematurity. The results appear to be promising, but further information is needed about the pertinent indications, the best dosage, timing, and route of administration of rHuEpo, as well as the appropriate method of iron supplementation. Patients with many other clinical disorders may benefit from rHuEpo therapy, but with the exception of the anemia of chronic renal failure the exact role of Epo in their management remains undefined at the present time.

**Key words:** erythropoietin, erythropoiesis, anemia of prematurity, infant child, recombinant human erythropoietin

Epo is heavily a glycosylated protein formed of 165 amino acids with a molecular mass of 34,000 Da. Except during fetal life, Epo is mostly produced by the kidney, but the exact cell of origin remains unknown. Ten percent of Epo production is also contributed by other organs, mainly the liver in which limited production capacity does not allow an adequate response to hypoxia. There are no preformed stores of Epo and any increase in the rate of production must be preceded by Epo gene transcription.<sup>1</sup> Epo production is regulated by a feedback mechanism by which the blood oxygen content is maintained at a constant level through the function of a kidney oxygen sensor.<sup>2</sup> The major determinant of Epo production is therefore the circulating red cell mass, but other factors also play a role, including kidney oxygen consumption, renal blood flow, oxygen saturation (mostly dependent on pulmonary function), and oxygen affinity of hemoglobin.

Epo exerts its action on target cells after binding to a specific Epo receptor whose structure and function have been extensively studied.<sup>3</sup> Both high-affinity and low-affinity receptors are present at the surface of erythroid cells (except mature red cells). Mouse and human placentas have low-affinity Epo receptors.<sup>4</sup> The peak receptor number is reached at the colony-forming unit-erythrocyte (CFU-E) stage, but Epo is necessary for the survival, proliferation, and differentiation of burst-forming unit-erythrocyte (BFU-E), CFU-E, and erythroblasts.

It is not the purpose of this review to detail the general physiology of Epo in man, which has been reviewed in other excellent articles.<sup>5,6</sup> Therefore, only the major features of Epo will be outlined here. We will discuss more thoroughly the specific aspects of Epo in infants and children, including the biology of the hormone and the therapeutic uses of recombinant human erythropoietin (rHuEpo). We will in particular address the pathophysiology and treatment of the anemia of prematurity.

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## PHYSIOLOGY OF ERYTHROPOIESIS AND ERYTHROPOIETIN IN THE FETUS AND NEONATE

Erythropoiesis undergoes a remarkable evolution throughout gestation and the neonatal period.<sup>7,8</sup> Hematopoiesis restricted to erythropoiesis begins in the yolk sac around day 14 of gestation. Fetal hematopoiesis begins primarily in the liver and secondarily in the spleen, between weeks 6 and 8 of gestation. Hepatic erythropoiesis decreases and marrow erythropoiesis increases during the second trimester, so that after birth the marrow remains the only erythropoietic organ.<sup>9</sup> Fetal erythropoiesis is controlled by Epo, particularly during the second half of gestation.<sup>10</sup> Epo receptors are found in mouse fetal liver.<sup>11</sup>

In several animal species, fetal Epo is synthesized primarily in the liver. Bilateral nephrectomy has no effect on the fetal Epo response to hypoxia induced by bleeding of pregnant goats.<sup>12</sup> In fetal sheep, it is the liver that has been shown to be the primary site of Epo formation until late in gestation.<sup>13,14</sup> The liver to kidney switch appears to begin in the third trimester and ends approximately 6 weeks after birth.<sup>14</sup> This explains why subtotal hepatectomy, but not bilateral nephrectomy, inhibited the Epo response to acute bleeding.<sup>13</sup> The liver to kidney switch appears to take place at the end of gestation in mice also, as fetal liver produces Epo up to day 18 of gestation but no longer by day 19 when Epo mRNA can be detected in the kidney in the presence of anemia.<sup>15</sup> The rat fetus appears to have extrahepatic Epo before day 17 of gestation, followed by hepatic and, to a lesser extent, renal Epo production, both of which become sensitive to maternal hypoxia during the last days of pregnancy.<sup>16</sup> The liver to kidney switch appears to take place after birth,<sup>17</sup> around day 10 in normal conditions, but as early as day 2 in hypoxic conditions.<sup>16</sup> Indirect evidence of the postnatal switch from liver to kidney Epo production is also available in humans. Infants born with severe renal disease have Epo levels and erythropoietic activities similar to those of normal infants.<sup>18</sup> A blunted Epo response to anemia in the fetus may be explained by the decreased sensitivity of the liver to hypoxia as compared with the kidney.<sup>19</sup>

## SERUM EPO IN ADULTS

In adults, serum Epo levels may vary considerably.<sup>6</sup> Levels are usually between 10 and 20 mU/ml in normal subjects, may decrease somewhat in primary polycythemia, but increase exponentially when anemia develops below Hct of 30–35%. Therefore, a serum Epo value must always be evaluated in relation to the degree of anemia. Serum Epo levels can be inappropriately high in secondary polycythemia, a feature permitting its diagnostic separation from primary polycythemia.<sup>20</sup> Epo levels inappropriately low for the

degree of anemia (Table I) are encountered not only in renal failure,<sup>21</sup> but also in a number of other conditions, including the anemia of chronic disorders.<sup>22</sup> Accordingly, inadequate Epo production has been found to contribute to the anemia of rheumatoid arthritis,<sup>23</sup> human immunodeficiency virus infection,<sup>24</sup> or cancer.<sup>25</sup> Intensive chemotherapy, whether followed by bone marrow transplantation or not, may cause a transient elevation of serum Epo levels.<sup>26,27</sup> However, in the long run, chemotherapy with cis-platinum<sup>28</sup> and allogeneic bone marrow transplantation<sup>26,27</sup> are associated with inappropriately low Epo levels, in part caused by renal impairment. Renal dysfunction is also a contributing factor for low Epo levels in multiple myeloma.<sup>29</sup> Serum Epo levels, although increased over nonpregnant values, remain relatively low for the degree of anemia in the first part of pregnancy but return progressively to adequate levels thereafter.<sup>30</sup>

## SERUM EPO IN FETUS AND NEONATE

Relatively low levels of immunoreactive Epo can be detected in fetal plasma from week 16 of pregnancy.<sup>31,32</sup> While a continuous increase of Hb is observed during gestation,<sup>31</sup> a correlation of Epo levels with gestational age was found in some<sup>32</sup> but not other<sup>31</sup> studies. Although this has been observed in some studies,<sup>33,34</sup> fetal Epo levels are usually not different in preterm as compared to term infants.<sup>35,36</sup> Labor can induce significant elevations of cord blood Epo levels<sup>37–40</sup> even if this has not been observed in all studies.<sup>41</sup> Altogether, fetal Epo levels in uncomplicated pregnancies before as well as after labor will usually not increase above 50 mU/ml.<sup>35,42,43</sup>

Neither during pregnancy<sup>32</sup> nor at birth<sup>36,43</sup> is there a significant correlation between serum Epo and Hb. However, when an anemia develops, as in the case of Rh immunization, a significant inverse relationship is evident.<sup>40,44,45</sup> In the case of diabetes mellitus, in which the hypoxia is not secondary to anemia, there is even a positive correlation between serum Epo and Hb that explains the polycythemia.<sup>46</sup> Although this is not ob-

TABLE I  
Conditions Associated with Defective  
Epo Production

Prematurity
Intrauterine transfusions
Chronic renal failure
Anemia of chronic disorder
Cancer (solid tumors, multiple myeloma)
Rheumatoid arthritis
Infectious diseases (AIDS)
Surgery
Chemotherapy with cisplatin
Allogeneic bone marrow transplantation
Early pregnancy

served in a normal uncomplicated pregnancy,<sup>32</sup> there is a good correlation between serum Epo and erythroblast counts in the case of chronic fetal hypoxia.<sup>44,46-49</sup>

At the end of pregnancy, either before labor or just after birth, there is usually a strong inverse correlation of serum Epo with cord blood pH,<sup>33,35,36,39,43-45,47,48</sup> but usually not with cord blood PaO<sub>2</sub>.<sup>32,35,43-45</sup> Accordingly, a number of situations have been associated with elevated fetal Epo levels (Table II). Hypertension with or without pre-eclampsia is one of them,<sup>39,43,50,51</sup> but elevated Epo levels after pre-eclamptic pregnancy rapidly resolve after birth.<sup>50</sup> Intravenous  $\beta$ -sympathomimetic tocolysis stimulates fetal Epo production, possibly by decreasing fetal oxygenation through alterations of metabolism and/or decreased placental blood flow.<sup>52</sup> Increased Epo levels are observed in fetuses with severe growth retardation.<sup>35,39,47</sup> Fetal distress causes a significant elevation of cord blood Epo,<sup>33,35,36,44,53</sup> even if this has not been shown in all studies.<sup>39</sup> This discrepancy may be due to the fact that, contrary to chronic hypoxia,<sup>35,47,50</sup> acute hypoxia was not always sufficient for inducing increased Epo synthesis.<sup>39</sup> While vasopressin and hypoxanthine are better indicators of acute hypoxia,<sup>39</sup> high Epo levels after normal pregnancies, but not after pre-eclampsia, indicate an increased risk of perinatal brain damage.<sup>51</sup> Maternal alcohol abuse is also accompanied by elevated fetal Epo levels that correlate with maternal alcohol intake, but it is not clear whether this is a direct effect of ethanol or is induced by chronic fetal hypoxia.<sup>53</sup>

Rh immunization results in fetal anemia from red blood cell destruction, and this anemia is partly compensated for by extramedullary hematopoiesis and erythroblastosis. Amniotic fluid and umbilical blood obtained by cordocentesis as well as at birth contain elevated Epo levels.<sup>40,44,45,48</sup> However, this increase in Epo production is minimal before week 20 or 24 of gestation<sup>40,48</sup> and takes place only in the presence of severe fetal anemia.<sup>48</sup> One can observe a strong inverse correlation between serum Epo and Hb, as well as a strong positive correlation between Epo and erythroblasts.<sup>40,44,45,48</sup> Elevated Epo levels can thus predict fetal distress and indeed correlate directly with fetal heart rate<sup>54</sup> and inversely with blood pH at birth.<sup>44,45,48</sup>

TABLE II  
Conditions Associated with Elevated Epo Levels  
at Birth

Labor
Intravenous $\beta$ -sympathomimetic tocolysis
Severe growth retardation
Maternal hypertension (with or without pre-eclampsia)
Maternal diabetes mellitus
Maternal alcoholism
Rhesus immunization (with anemia)

Maternal diabetes mellitus causes fetal polycythemia and thrombocytopenia in proportion to the maternal glycosylated Hb percentage.<sup>55</sup> Although the number of CFU-E may be increased,<sup>56</sup> the number of BFU-E and their growth in the presence of Epo appear to be normal.<sup>56,57</sup> It is thus suggested that polycythemia is an adaptive response in infants of diabetic mothers. Maternal hyperglycemia induces fetal hyperglycemia, which in turn stimulates insulin secretion by the fetus. The resulting increased metabolism causes tissue hypoxia and acidemia that are thought to be responsible for increased Epo production.<sup>46,49,58</sup> Indeed fetal Epo correlates directly with fetal insulin.<sup>49</sup> Antepartum control of maternal hyperglycemia is critical, as elevated fetal Epo levels are observed in patients with poorly controlled diabetes<sup>46,49,58</sup> but not in patients with good glycemic control.<sup>57</sup> However, not all the elevation of erythropoietic activity can be accounted for by increased Epo production and other factors could contribute, including insulin itself.<sup>46,56</sup>

## SERUM EPO IN CHILDREN

While serum Epo levels may be increased at birth, they decrease rapidly in the following hours in infants without postnatal hypoxia.<sup>50</sup> The lowest Epo values are reached during the first 2 months after birth.<sup>33</sup> Thereafter, serum Epo values increase slightly and remain constant at levels very similar to those observed in adults,<sup>33,59</sup> although some difference has occasionally been noted between children aged 1 to 2 years and children aged 4 years or older.<sup>60</sup> There appears to be no correlation between Hb and serum Epo in healthy children,<sup>59</sup> but this is not unexpected for subjects with Hb values within the normal range. Inadequate Epo production may be encountered in a variety of conditions (Table I). Similarly to adults, children with chronic renal failure have serum Epo levels within the normal range or slightly increased, but inappropriately low for the degree of anemia.<sup>61-63</sup> While nondialyzed patients had the highest Hb,<sup>61</sup> those on peritoneal dialysis had intermediate levels that were significantly higher than those in hemodialysis patients because of somewhat better Epo production.<sup>63</sup> Epo production is further reduced, but certainly not abrogated, in anephric children, underlining the role of extrarenal sites in Epo production.<sup>62</sup>

Serum Epo levels correlate inversely with Hb in children with hematological disorders, including aplastic anemia, transient erythroblastopenia, iron deficiency anemia, or thalassemia.<sup>33,64</sup> The slope of the regression may be steeper in iron deficiency anemia than in marrow erythroblastopenia or pancytopenia, possibly because the presence of erythroid progenitors could limit the elevation of serum Epo in children with less severe iron deficiency anemia.<sup>64</sup> This inverse relationship was maintained in children with acute leukemia, in whom

treatment with high-dose chemotherapy produced further transient increments of serum Epo levels beyond values expected for the degree of anemia.<sup>65</sup> However, children with solid tumors have been found to have inadequate Epo production in response to anemia.<sup>66</sup>

During the first 4 months of life, term infants with cyanotic heart disease have significantly higher serum Epo concentrations than normal adults and Epo levels do not correlate with Hb, arterial oxygen contents, or PaO<sub>2</sub>.<sup>67</sup> However, older children with cyanotic or acyanotic congenital heart disease have similar serum Epo levels, comparable to those found in normal adults.<sup>33,68</sup> There was a significant negative correlation of serum Epo with arterial oxygen content but not with Hb or PaO<sub>2</sub>.<sup>33,68</sup> Therefore, cyanotic children appear to initially increase Epo production in response to hypoxia until stimulated erythropoiesis increases Hb to values ensuring the return of Epo levels to normal.<sup>68</sup>

### THERAPEUTIC USES OF rHuEPO IN ADULTS

After cloning of the gene for Epo, rHuEpo has become available for therapeutic use (Table III).<sup>69</sup> It has first been shown that rHuEpo could correct the anemia of chronic renal failure and eliminate the need for transfusion.<sup>70</sup> Besides this purely substitutive therapy, a number of clinical disorders more or less characterized by defective Epo production have been shown to benefit from treatment with rHuEpo. However, the doses necessary to achieve responses in patient with acquired immune deficiency syndrome (AIDS),<sup>71</sup> rheumatoid arthritis,<sup>72</sup> multiple myeloma,<sup>73</sup> or other forms of cancer<sup>74</sup> are usually much higher than those used in hemodialysis patients. The same is true for patients

undergoing chemotherapy,<sup>74</sup> radiotherapy,<sup>75</sup> or bone marrow transplantation.<sup>76</sup> The efficacy of Epo in treating the anemia of myelodysplastic syndromes is limited by the ineffectiveness of erythropoiesis in these disorders, whether rHuEpo is used alone<sup>77</sup> or in association with granulocyte colony-stimulating factor (CSF) or other growth factors.<sup>78</sup> rHuEpo has also been proposed in the treatment of sickle cell anemia to increase fetal hemoglobin levels.<sup>79</sup> There is also a place for rHuEpo in the stimulation of normal erythropoiesis before elective surgery to avoid transfusions or to increase the preoperative collection of autologous blood<sup>80</sup>, or bone marrow donation.<sup>81</sup>

### TREATMENT OF THE ANEMIA OF CHRONIC RENAL FAILURE IN CHILDREN

As in adults, anemia is a prominent feature of chronic renal failure. In addition to the usual symptoms observed in adults, this anemia also causes retarded growth and delayed neurological development in children.<sup>82</sup> The severity of the anemia is inversely related to the glomerular filtration rate.<sup>83</sup> The pathophysiological features of the anemia of chronic renal failure in children are very similar to those in adults and are mostly due to inappropriate Epo production.<sup>61-63</sup> However, the severity of renal anemia is more pronounced in children for several reasons, including the absence of androgen,<sup>82</sup> larger blood losses by gastrointestinal bleeding,<sup>84</sup> and proportionally more significant blood losses in the hemodialysis circuit.<sup>84</sup>

Treatment with rHuEpo has been shown to correct the anemia of chronic renal failure and eliminate the need for transfusion in adult patients.<sup>70</sup> rHuEpo is also

TABLE III  
Potential Indications for rHuEpo Therapy in Children

Indication	References in Adults	References in Children
Neonatal anemias		
Anemia of prematurity		126,139-148
Anemia of bronchopulmonary dysplasia		98
Anemia after intrauterine transfusion for Rhimmunization		96,97
Anemias of infancy and childhood		
Anemia of chronic renal failure	70	85-88,94
AIDS anemia	71	
Anemia of rheumatoid arthritis	72	100
Anemia of cancer	73,74	
Iatrogenic anemias of infancy and childhood		
Postchemotherapy anemia	74,75	105
Postoperative anemia	80	101,102
Postmarrow transplantation anemia	76	103,104
Other indications		
Sickle cell anemia	79	
Congenital heart diseases		
Autologous blood donation	80	
Bone marrow donation	81	81

effective in children on hemodialysis<sup>85</sup> or peritoneal dialysis.<sup>86-88</sup> Epo can be given intravenously usually three times weekly,<sup>85</sup> subcutaneously either three times<sup>88</sup> or twice<sup>87</sup> or once<sup>86</sup> a week, or even intraperitoneally.<sup>89</sup> The initial dose is between 75 and 225 U/kg/wk or probably a little less when the subcutaneous route is used. The increase in Hct is also probably in part contributed by decreased blood losses because of increased platelet counts and function.<sup>90</sup> Once the target Hct has been reached, the dose can usually be reduced by one-third to one-half. However, occasional patients will need much higher doses. For instance after nephrectomy for congenital nephrotic syndrome, the response may be very poor because growth after nephrectomy may be so rapid that even Epo-stimulated erythropoiesis is not able to keep up with it.<sup>91</sup> Correction of the anemia produces marked improvement of cardiac performance<sup>92</sup> and exercise tolerance,<sup>85</sup> which translates into enhanced ability to engage in activities.<sup>86</sup> However, little or no effect on the growth rate of these children has been noted.<sup>93</sup> The side effects of rHuEpo are the same as in adults, the most prominent being *de novo* or worsened hypertension.<sup>94</sup> As in adults, the adequacy of iron supplementation is critical to the success of therapy.<sup>70</sup>

#### OTHER USES OF RHUEPO IN INFANTS AND CHILDREN

Patients with a number of other conditions may benefit from treatment with rHuEpo (Table III). However most reports consisted of anecdotal cases or uncontrolled studies. Although these indications appear to be promising, no general recommendations can be made at the present time.

Multiple intravascular intrauterine transfusions for Rh-immunization suppress prenatal and postnatal erythropoietic activity by limiting Epo production and this may result in severe neonatal anemia.<sup>95</sup> This aregenerative anemia appearing in the 1st to 3rd month of life may respond adequately to treatment with 100-200 U/Kg/d or rHuEpo.<sup>96,97</sup> Patients with the anemia of bronchopulmonary dysplasia are not all born prematurely but most of them will respond to 200 U/Kg/d of rHuEpo with increases of reticulocyte counts and Hct values.<sup>98</sup>

RHuEpo given at doses up to 2000 U/Kg/d is ineffective in children with Diamond-Blackfan anemia.<sup>99</sup> However, provided iron supplements are given, the response of the anemia associated with active juvenile rheumatoid arthritis to about 300 U/Kg/wk of rHuEpo may be impressive.<sup>100</sup>

RHuEpo may also be used to increase red blood cell production in surgery. Very high doses of 500 U/Kg/d have been used in children with burned injuries<sup>101</sup> but only 40-80 U/Kg/wk were required for efficacy in children with persisting aregenerative ane-

mia after cardiac transplantation.<sup>102</sup> The use of rHuEpo at doses of 450 U/Kg/wk could be particularly interesting to increase Hct in children undergoing bone marrow donation.<sup>81</sup> As Epo deficiency may occur after allogeneic bone marrow transplantation, delayed erythroid engraftment may be treated efficiently with rHuEpo.<sup>103</sup> When Epo is already given at a dose of 75 U/Kg/d immediately after transplant, erythroid repopulation is considerably accelerated without detrimental effect on myeloid and platelet engraftment.<sup>104</sup> Finally, cisplatin chemotherapy may be complicated by Epo deficiency if a significant degree of renal failure develops and the subsequent anemia may respond to rHuEpo.<sup>105</sup>

Although this has only been tested in adults with sickle cell anemia, the combination of rHuEpo and hydroxyurea may decrease the incidence of vaso-occlusive crises by increasing HbF synthesis.<sup>79</sup> Hydroxyurea could abrogate the polycythemic effect of rHuEpo, which in turn could limit the marrow toxicity of hydroxyurea.<sup>9</sup>

In infants with congenital heart disease and left-to-right shunts, the normal decline in Hct after birth results in congestive heart failure and poor growth. As increasing the Hb concentration is associated with reduced signs of left-to-right shunt,<sup>106</sup> therapy with rHuEpo may therefore bring sufficient clinical improvement to defer surgery until the child reaches an ideal weight, but this remains to be demonstrated.<sup>9,107</sup>

#### PHYSIOPATHOLOGY OF THE ANEMIA OF PREMATURITY

Among neonates with birth weight <1250 g, 90% require blood transfusions, on average five, and the problem is growing because of the improving survival of these infants.<sup>108</sup> It is very difficult to determine the precise indication for blood transfusion in premature infants, as classical signs of anemia may not be as relevant.<sup>108-110</sup>

There are two types of anemia. The first occurs during the first 2 weeks after birth in sick, often ventilated, infants requiring intensive care and multiple blood tests.<sup>110,111</sup> Because of these severe blood losses and the time necessary for rHuEpo to become active, treatment is less likely to reduce the number of transfusions during this period.<sup>110,111</sup> This is a problem because of the increased risks associated with blood transfusion in premature infants, including viral and bacterial infections, graft-vs.-host disease, and immune problems.<sup>109,111-113</sup> A second type of anemia, which is called the anemia of prematurity, occurs at around 6 weeks after birth and is characterized by decreased Hb and a failure to compensate by increasing erythropoiesis. Indeed, it may be considered as an exaggeration of the normal fall of Hb that all infants experience after birth.<sup>108,111,114,115</sup>

The mean number of circulating BFU-E per nucleated cells between the 19th and 30th week of gestation is 3 times higher than in cord blood at birth and 10 times higher than in adult bone marrow.<sup>31</sup> The number of BFU-E in anemic preterm infants is higher than in nonanemic preterm infants or in adults.<sup>116</sup> Sensitivity to Epo is highest in fetal, intermediate in newborn, and lowest in adult cultures.<sup>117</sup> Cord blood BFU-E<sup>116,118</sup> and marrow CFU-E<sup>119</sup> from infants with the anemia of prematurity are at least as responsive to Epo as are progenitors from healthy neonates or adults. Therefore, the anemia of prematurity does not originate from abnormalities in the number or function of erythroid progenitors.

The pathophysiology of the anemia of prematurity is complex (Table IV), but one of the main features is a defect in the capacity to increase Epo synthesis in response to anemia. Although serum Epo levels are normal or even increased at birth, Epo production decreases considerably during the first weeks after birth in term as well as preterm infants and remains low in preterm infants until 6 to 10 weeks before re-increasing to levels similar to those observed in adults.<sup>34,109,115,120-123</sup> Epo levels are not only inversely related to Hb concentration but also depend on its oxygen unloading capacity<sup>109,115,120,122,123</sup> so that the best correlation is seen with the central venous oxygen tension.<sup>123</sup> Therefore, because the Epo response to relative hypoxia is based on available oxygen, it remains inadequate for the degree of anemia.<sup>118,120-126</sup> The greatest fall in Hb after birth is observed in the most immature infants,<sup>115</sup> and the Epo response to anemia is blunted to a greater extent in the youngest premature infants.<sup>120</sup> There are a number of reasons for this inappropriate Epo production. Epo production is down-regulated by the sudden delivery to an atmosphere that is relatively hyperoxic compared with the intrauterine milieu.<sup>126</sup> During fetal life, the relatively insensitive hepatic sen-

sor may be sufficient to regulate Epo production in the hypoxic conditions of low arterial PO<sub>2</sub> and high percentage of fetal hemoglobin.<sup>108</sup> After premature delivery and the establishment of an adult-like circulation with a large increase in pulmonary blood flow and arterial PO<sub>2</sub>, the hepatic oxygen sensor will shut down Epo release until infants reach the age at which renal production starts.<sup>108</sup> The Hb-oxygen dissociation curve at birth is shifted to the left because of the high proportion of HbF and low concentration of functional 2,3-diphosphoglycerate.<sup>109,115</sup> Later, when an increasing proportion of HbA and higher concentration of 2,3-diphosphoglycerate are produced by the neonate, the ensuing reduction in oxygen affinity will further depress Epo production.<sup>122</sup> Blood transfusions will worsen the phenomenon by increasing the total Hb level as well as the proportion of HbA.<sup>109,115</sup> After cessation of transfusions, a temporary rise in HbF is seen,<sup>127</sup> which may ameliorate the Epo response.

A number of other factors may contribute to the anemia of prematurity. As the Hct increases during fetal life, premature infants will have lower values than term infants.<sup>31</sup> Urgent resuscitation maneuvers may not permit optimal placentofetal transfusion at birth.<sup>126</sup> This will also reduce the number of circulating erythropoietic progenitors in the preterm newborn.<sup>81</sup> Short erythrocyte life span in the neonate will further be reduced in preterm infants by a particular sensitivity to oxidative stress.<sup>126</sup> Blood sampling for laboratory tests may be considerable: an 800-g infant will lose half of the blood volume if 5 to 6 ml of blood is removed daily during the 1 week.<sup>108,115</sup> As the premature infant has a very rapid growth rate, the ensuing expansion of plasma volume will further dilute red blood cells.<sup>126</sup>

This accelerated growth requires a rapid expansion of the red cells mass and large amounts of iron are thus needed. Because the fetus accumulates iron throughout pregnancy,<sup>128</sup> iron stores are less adequate in preterm infants and particularly in small-for-age premature newborns.<sup>129</sup> Although the relatively immature gastrointestinal tract appears to absorb iron well,<sup>130</sup> stores may be rapidly exhausted by the enormous demand for iron associated with rapid growth and by the negative net iron balance presumably due to high fecal losses.<sup>130</sup> Net iron balance will be positive and iron deficiency prevented only if sufficient oral or intramuscular iron supplements are provided early in life.<sup>129-132</sup>

TABLE IV

#### Physiopathology of the Anemia of Prematurity

Decreased Hct at birth
Interrupted Hct increase during fetal life
Less than optimal placentofetal transfusion at birth
Loss of red blood cells after birth
Shorter erythrocyte life span
Blood sampling for blood tests
Defective Epo production
Sudden delivery to hyperoxic environment
Adult-type circulation sending oxygenated blood to poorly sensitive hepatic oxygen sensor
Liver to kidney switch of Epo production not achieved
Transfusions
Very rapid growth rate
Rapidly expanding red cell mass
Hemodilution
Limited iron supply

#### TREATMENT OF THE ANEMIA OF PREMATURITY

Administration of rHuEpo to rhesus monkeys induced significant increases of hemoglobin in adults but no change in Hb despite increased reticulocytosis in infant monkeys.<sup>133</sup> This may be due in part to the larger volume of distribution, greater clearance and shorter half-life and residence times in infant compared with adult

animals.<sup>133,134</sup> This has also been shown in man.<sup>135</sup> The Epo disappearance rate after birth in neonates without hypoxia is faster than in adults.<sup>50</sup> Another critical factor is iron, which is clearly a rate-limiting factor in erythropoiesis during the neonatal period<sup>136</sup> and even more so during treatment with Epo.<sup>133</sup>

There has been much interest in the use of rHuEpo as an alternative to blood transfusion in infants with the anemia of prematurity (Tables V and VI).<sup>107,111,137,138</sup> A European randomized open-label study compared 50 untreated children with 43 infants treated with 70 U/kg/wk of rHuEpo. This low dose given for 3 weeks starting on day 4 after birth did not produce any effect on erythropoiesis.<sup>139</sup> An American study of premature infants receiving between 10 and 200 U/kg/wk of rHuEpo for 4 weeks starting at a median time of 42 days after birth obtained increased reticulocyte counts and Hct in most infants, with 25% of them still requiring blood transfusions. However, these results are very difficult to interpret because there was no control group, and the observed increases were not dose-dependent.<sup>140</sup> Halperin and co-workers<sup>126,141</sup> administered 75, 150, 300, or 600 U/kg/wk of rHuEpo for 4 weeks starting at a median time of 27 days after birth. There was a dose-dependent elevation of reticulocyte counts and a stabilization of Hct, with only few patients requiring transfusions. In comparison, Hct fell and blood transfusions were necessary in 20% of 66 historical control subjects. Shannon *et al.*<sup>142</sup> randomly treated infants with 200 U/kg/wk of rHuEpo or placebo for 6 weeks starting at a median time of 22 days after birth. Reticulocytes increased earlier, but otherwise there were no differences in reticulocyte counts, Hct, or transfusion needs between the two groups.<sup>142</sup> The same group later randomly assigned infants to either placebo or 500 U/kg/wk of rHuEpo, with the dose being increased to 1000 U/kg/wk after 2 weeks if target reticulocyte counts were not achieved. Treatment was given for 6 weeks starting on day 10 to 35 after birth. Reticulocytes increased significantly more in the treated group, Hct remained stable in the treated group but fell in the placebo group, and transfusions were less required in treated versus placebo infants.<sup>143</sup> In a randomized open-label study, Ohls and Christensen<sup>144</sup> compared the efficacy of transfusions with that of 700 U/kg/wk of rHuEpo for 3 weeks starting at a median time of 41 days after birth. As expected, transfusions were more efficacious in rapidly rising the Hct, but reticulocytes increased more in the rHuEpo-treated group and at the end of the study, the Hct was very similar in the two groups. Carnielli *et al.*<sup>145</sup> randomly assigned infants to receive either no treatment or 1200 U/kg/wk of rHuEpo from day 2 after birth until discharge. Contrary to other studies, control subjects were not given iron supplements. Reticulocytes and Hct fell in control subjects, while reticulocytes increased more and Hct stabilized in the treated group, resulting in

fewer transfusions administered. However, the criteria for ordering red cell transfusion were very liberal. Emmerson *et al.*<sup>146</sup> conducted a randomized double-blind study comparing placebo with rHuEpo at the dose of 100, 200, or 300 U/kg/wk from day 7 after birth until discharge. There were definite signs of more active erythropoiesis in infants receiving rHuEpo. Approximately twice as many infants receiving placebo required red cell transfusions, resulting in the end in similar declines of Hct values throughout the study in the two groups. Bechensteen *et al.*<sup>147</sup> compared no treatment with 300 U/kg/wk of rHuEpo for 4 weeks starting on day 21 after birth. Compared with control subjects treated patients had higher reticulocyte counts and maintained their Hct, and none (vs. 4 of 15 control subjects) required red cell transfusions.<sup>147</sup> In a large study conducted by Messer *et al.*<sup>148</sup> infants were consecutively assigned to three treatment groups receiving 300, 600, or 900 U/kg/wk of rHuEpo for 6 weeks starting on day 10 after birth. Infants whose parents did not consent to the study served as control subjects. Reticulocytes increased more and in a dose-dependent fashion in treated infants, while Hct decreased less albeit without a clear dose-response effect. This resulted in significantly fewer blood transfusions.

Many differences among these clinical trials make comparison between them and definite conclusions difficult to derive. Smaller infants are sicker, and thus probably less responsive to rHuEpo and require more blood sampling for laboratory tests, which may hide the beneficial effect of treatment.<sup>142,143</sup> However, as they receive the largest number of transfusions, the smallest infants are the most likely to benefit from effective therapy.<sup>138</sup> Liberal transfusion criteria will overstate the benefit of rHuEpo, not only because more transfusions will be given, but also because repeated transfusions will suppress the endogenous Epo response to anemia.<sup>145</sup> As the anemia of prematurity is an exaggeration of the fall of hemoglobin that all infants undergo during the first month of life,<sup>115</sup> it should not be expected that treatment in the first week after birth would increase the Hct<sup>139,145,146,148</sup> but would only slow its fall,<sup>145,146,148</sup> while late treatment could produce significant increments.<sup>140,144</sup> Doses <300 U/kg/wk will not bring about significant changes in erythropoietic activity.<sup>139-142</sup> Establishing the appropriate dose and route of supplementary iron remains an open question. The highest doses of iron supplements are associated with the best responses to rHuEpo.<sup>141,143,147,148</sup> We do not know what dose of iron can be safely administered enterally or intravenously to premature babies and whether treatment should be discontinued if storage iron falls below some critical level because iron is absolutely required for cell division in many organs.<sup>138</sup>

The tolerance to rHuEpo has been excellent and no serious side effects have been attributed to treatment. Although a significant decrease in neutrophil counts

TABLE V  
Treatment of the Anemia of Prematurity with rHuEpo: Study Characteristics

Author	Year	No. treated	Gestational age (wk)*	Age (days)*	Dose (U/kg/wk)	Route	Duration (wk)	Oral iron (mg/kg/day)	Design
Obladen	1991	43	30 (28–32)	4	70	SC	3	2	Randomized, open-label trial (50 controls)
Beck	1991	16	29 (27–32)	42 (25–59)	10–200***	IV	4	3	Open-label trial (no controls)
Halperin	1992	18	31 (28–33)	27 (21–33)	75–600***	SC	4	2–8**	Open-label Trial (66 historical controls)
Shannon	1991	10	27 (<33)	22 (10–35)	200	IV	6	3	Randomized, placebo-controlled trial (10 controls)
Shannon	1992	4	27 (25–29)	19 (8–28)	500–1000***	SC	6	3–6****	Randomized, placebo-controlled trial (4 controls)
Ohls	1991	10	28 (26–30)	41 (21–70)	700	SC	3	2	Randomized to received transfusion ( $n = 9$ ) or rHuEpo
Carnielli	1992	11	30 (25–32)	2	1200	IV	To discharge	3 <sup>§</sup>	Randomized, open-label trial (11 controls)
Emmerson	1993	15	30 (27–33)	7	100–300	SC	To discharge	6	Randomized, placebo-controlled trial (8 controls)
Bechensteen	1993	14	30 (27–31)	21	300	SC	4	18	Randomized, open-label trial (15 controls)
Messer	1993	31	30 (<33)	10	300–900	SC	6	3–15†	Open-label trial (20 concomitant controls)

\*Mean (range).

\*\*\*Dose escalation in groups of infants.

\*\*\*†Dose escalation in same patients.

§IV.



TABLE VI  
Treatment of the Anemia of Prematurity with rHuEpo: Results

Author	Year	Reticulocytes	Hb/Hct	Transfusions (No. transfused/ No. evaluable)	Ferritin	SeFe or Tf saturation	Neutrophils	Platelets	Comments
Obladen	1991	Stable	Decrease in both groups	23/45	Stable	NA	Stable	Increase in both groups	
Beck	1991	Non-dose-dependent increase	Non-dose-dependent increase	4/16	Decrease	Decrease	Transient decrease in some	Mild increase	
Halperin	1992	Dose-dependent increase	Stable in rHuEpo, decrease in controls	3/18	Decrease	Decrease	Late decrease in some	Transient increase	Increased HbF; stable BFU-E; nonsignificant decrease of CFU-GM
Shannon	1991	Earlier increase in rHuEpo	Decrease in both groups	6/10 (8/10 in controls)	NA	NA	Stable	Stable	No effect on HbF due to large transfusions
Shannon	1992	Larger increase in rHuEpo	Stable in rHuEpo; decrease in controls	1/4 (3/4 in controls)	Decrease	Stable	Stable	Stable	Increased HbF
Ohls	1991	Larger increase in rHuEpo	Faster increase in transfused		Decrease	NA	Decrease more in rHuEpo	Stable	Increased marrow erythroblasts and CFU-E; stable BFU-E and CFU-GM
Carnielli	1992	Larger increase in rHuEpo	Stable in rHuEpo; decrease in controls	0.8 per infant (3.1 in controls)	NA	NA	Stable	Stable	
Emmerson	1993	Larger increase in rHuEpo	Decrease in both groups	7/15 (7/8 in controls)	Decrease	NA	Stable	Stable	Increased HbF
Bechensteen	1993	Larger increase in rHuEpo	Stable in rHuEpo; decrease in controls	0/14 (4/15 in controls)	Decrease	Decrease	Stable	Stable	
Messer	1993	Dose-dependent increase	Smaller decrease in rHuEpo	6/31 (9/20 controls)	NA	Decrease	Stable	Stable	

SeFe, serum iron; Tf, transferrin; NA, not available.

has been observed in uncontrolled studies,<sup>140,141,144</sup> this complication has not been encountered in larger or randomized trials.<sup>139,142,145,147,148</sup> Administration of very large doses of Epo to newborn rats resulted in reduced numbers of marrow and spleen CFU-granulocyte-macrophage and diminished neutrophil production.<sup>149</sup> However, studies in serum-free culture systems in the presence of interleukin 3, granulocyte-macrophage CSF, and granulocyte CSF alone or in combination, provided no evidence for a detrimental effect of Epo on myelopoiesis.<sup>150</sup>

## CONCLUSIONS ON THE USE OF rHuEPO IN INFANTS AND CHILDREN

Many indications for rHuEpo accepted in adults may also be valid in children. Provided that adequate iron supplements are given, erythropoietin at doses of 100 to 300 U/Kg/wk is very effective in correcting the anemia of chronic renal failure in patients on dialysis or not. It is now clear that rHuEpo is very effective in stimulating erythropoietic activity in infants with the anemia of prematurity. Whether this stimulation of erythropoiesis will translate into increased Hct and a reduction in transfusion needs depends on the age of the infants, the dose used (at least 300 U/Kg/wk), the adequacy of iron supplementation (at least 6 mg/kg/day), and the importance of concomitant blood sampling for laboratory tests. Despite interesting efficacy in some pilot studies, the limited experience obtained so far precludes general recommendations for the use of rHuEpo in other forms of anemia, such as the late postnatal anemia caused by intrauterine transfusions, the anemia of bronchopulmonary dysplasia, the anemia of chronic juvenile arthritis, the anemia associated with bone marrow donation or transplantation or with burn injuries or surgery. The role of rHuEpo in increasing the Hct of infants with congenital heart disease and left-to-right shunt or in stimulating the production of fetal Hb in children with sickle cell anemia should be investigated. Unless the critical role of adequate iron supplementation is recognized, absolute functional iron deficiency will limit the efficacy of any treatment with rHuEpo. In that regard intravenous iron will be more efficient than orally administered iron, but its safety in children is not yet well established.

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